

Atty Dkt. No.: 10981620-3
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AMENDMENTS

In the claims:

50. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of target nucleic acids with a collection of substrate bound probe nucleic acid features that includes at least one background nucleic acid feature that is an empirically observed inactive probe that does not hybridize to a fully complementary fluorescently labeled target nucleic acid as determined in an assay wherein said probe is provided in an array that is contacted with said fluorescently labeled fully complementary target under said hybridization conditions, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted collection are 50% melted;

(b) separating unbound target nucleic acids/label from said collection of probe nucleic acid features;

(c) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said collection of probe nucleic acid features~~; and

(d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature;

wherein said method is further characterized by including a target nucleic acid labeling step prior to said detecting step(c).

51. (Previously Presented) The hybridization assay according to Claim 50, wherein said sample of target nucleic acids is labeled with a detectable label prior to said contacting step.

52. (Previously Presented) The hybridization assay according to Claim 50, wherein said sample of target nucleic acids is labeled with a detectable label between said contacting and detecting steps.

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53. (Canceled)

54. (Canceled)

55. (Previously Presented) The method according to Claim 50, wherein said collection of substrate bound probe nucleic acid features is an array of nucleic acid features.

56. (Previously Presented) The method according to Claim 50, wherein said hybridization assay is a method of estimating the background noise in a hybridization assay.

57. (Previously Presented) The method according to Claim 50, wherein said method is a method of validating a test background feature.

58. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of target nucleic acids with a collection of substrate bound probe nucleic acid features that includes at least one background nucleic acid feature, wherein said at least one background feature is made up of a probe nucleic acid selected from the group consisting of SEQ ID NOS: 05 to 32, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted collection are 50% melted;

b) separating unbound target nucleic acids/label from said collection of probe nucleic acid features;

(c) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said collection of probe nucleic acid features~~; and

(d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from at said ~~at said~~ least one other probe nucleic acid feature;

wherein said method is further characterized by including a target nucleic acid labeling step prior to said detecting step(c).

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59. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of target nucleic acids with a collection of substrate bound probe nucleic acid features that includes at least one background nucleic acid feature, wherein said at least one background feature is made up of a probe nucleic acid that is chosen from: (i) a probe nucleic acid that forms a stable intramolecular structure; (ii) a probe nucleic acid that comprises reverse polarity nucleotide analogs; and (iii) a probe nucleic acid that comprises abasic phosphodiester, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted collection are 50% melted;

b) separating unbound target nucleic acids/label from said collection of probe nucleic acid features;

(c) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said collection of probe nucleic acid features~~; and

(d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature;

wherein said method is further characterized by including a target nucleic acid labeling step prior to said detecting step(c).

60. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of detectably labeled target nucleic acids with an array of probe nucleic acid features that includes at least one background nucleic acid feature that is an empirically observed inactive probe that does not hybridize to a fully complementary fluorescently labeled target nucleic acid as determined in an assay wherein said probe is provided in an array that is contacted with said fluorescently labeled fully complementary target under said hybridization conditions, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted array are 50% melted;

(b) separating non-hybridized target nucleic acids/label from said array;

(c) detecting a signal, if present, from said at least one background

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~~feature and at least one other probe nucleic acid feature - the presence of~~

~~target nucleic acids hybridized to said array probe nucleic acid features; and~~

(d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature;

wherein said method is further characterized by including a target nucleic acid labeling step prior to said detecting step(c).

61. (Canceled)

62. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of detectably labeled target nucleic acids with an array of probe nucleic acid features that includes at least one background nucleic acid feature, wherein said at least one background feature is made up of a probe nucleic acid selected from the group consisting of SEQ ID NOS: 05 to 32, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted array are 50% melted;

(b) separating non-hybridized target nucleic acids from said array; and

(c) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature - the presence of target nucleic acids hybridized to said array probe nucleic acid features; and

(d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature.

63. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of detectably labeled target nucleic acids with an array of probe nucleic acid features that includes at least one background nucleic acid feature, wherein said at least one background feature is made up of a probe nucleic acid that is chosen from: (i) a probe nucleic acid that forms a stable intramolecular structure; (ii) a probe nucleic acid that comprises reverse polarity nucleotide analogs; and (iii) a probe nucleic acid that comprises abasic phosphodiester, wherein said contacting occurs between 10 - 25°C below the

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average temperature (T_m) at which nucleotide hybrids of the contacted array are 50% melted;

- (b) separating non-hybridized target nucleic acids from said array;
- (c) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said array probe nucleic acid features~~; and
- (d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature.

64. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of target nucleic acids with an array of probe nucleic acid features that includes at least one background nucleic acid feature that is an empirically observed inactive probe that does not hybridize to its fully complementary target nucleic acid as determined in an assay wherein said probe is provided in an array that is contacted with said fluorescently labeled fully complementary target under said hybridization conditions, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted array are 50% melted;

- (b) separating non-hybridized target nucleic acids from said array;
- (c) detectably labeling target nucleic acids hybridized to said array of probe nucleic acid features;
- (d) separating unbound label from said array;
- (e) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said array of probe nucleic acid features~~; and
- (f) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature.

65. (Canceled)

66. (Currently Amended) A hybridization assay comprising:

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- (a) contacting a sample of target nucleic acids with an array of probe nucleic acid features that includes at least one background nucleic acid feature, wherein said at least one background feature is made up of a probe nucleic acid selected from the group consisting of SEQ ID NOS: 05 to 32, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted array are 50% melted;
- (b) separating non-hybridized target nucleic acids from said array;
- (c) detectably labeling target nucleic acids hybridized to said array of probe nucleic acid features;
- (d) separating unbound label from said array;
- (e) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said array of probe nucleic acid features~~; and
- (f) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature.

67. (Currently Amended) A hybridization assay comprising:

- (a) contacting a sample of target nucleic acids with an array of probe nucleic acid features that includes at least one background nucleic acid feature, wherein said at least one background feature is made up of a probe nucleic acid that is chosen from: (i) a probe nucleic acid that forms a stable intramolecular structure; (ii) a probe nucleic acid that comprises reverse polarity nucleotide analogs; and (iii) a probe nucleic acid that comprises abasic phosphodiester, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted array are 50% melted;
- (b) separating non-hybridized target nucleic acids from said array;
- (c) detectably labeling target nucleic acids hybridized to said array of probe nucleic acid features;
- (d) separating unbound label from said array;
- (e) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said array of probe nucleic acid features~~; and

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(f) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature.

68. (Canceled)

69. (Canceled)

70. (Canceled)

71. (Currently Amended) A hybridization assay comprising:

(a) contacting a sample of target nucleic acids with a collection of substrate bound probe nucleic acid features that includes at least one background nucleic acid feature made up of background probes that do not selectively bind to any of said target nucleic acids, wherein said contacting occurs between 10 - 25°C below the average temperature (T_m) at which nucleotide hybrids of the contacted collection are 50% melted;

(b) washing said contacted array to remove unbound target nucleic acids/label from said array;

(c) detecting a signal, if present, from said at least one background feature and at least one other probe nucleic acid feature ~~the presence of target nucleic acids hybridized to said collection of probe nucleic acid features~~; and

(d) subtracting a detected signal from said at least one background nucleic acid feature from signal detected from said at least one other probe nucleic acid feature;

wherein said method is further characterized by including a target nucleic acid labeling step prior to said detecting step(c).

72. (Previously Presented) The hybridization assay according to Claim 71, wherein said sample of target nucleic acids is labeled with a detectable label prior to said contacting step.

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73. (Previously Presented) The hybridization assay according to Claim 71, wherein said sample of target nucleic acids is labeled with a detectable label between said contacting and detecting steps.
74. (Canceled)
75. (Canceled)
76. (Previously Presented) The method according to Claim 71, wherein said collection of substrate bound probe nucleic acid features is an array of nucleic acid features.
77. (Previously Presented) The method according to Claim 71, wherein said hybridization assay is a method of estimating the background noise in a hybridization assay.
78. (Previously Presented) The method according to Claim 71, wherein said method is a method of validating a test background feature.
79. (Previously Presented) The method according to Claim 59, wherein said stable intramolecular structure is a hairpin.
80. (Previously Presented) The method according to Claim 59, wherein said stable intramolecular structure is a pseudo half knot.
81. (Previously Presented) The method according to Claim 63, wherein said stable intramolecular structure is a hairpin.
82. (Previously Presented) The method according to Claim 63, wherein said stable intramolecular structure is a pseudo half knot.
83. (Previously Presented) The method according to Claim 67, wherein said stable intramolecular structure is a hairpin.

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84. (Previously Presented) The method according to Claim 67, wherein said stable intramolecular structure is a pseudo-half knot.